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Effect of Increased CRM_{197} Carrier Protein Dose on Meningococcal C Bactericidal Antibody Response

Lucia H. Lee and Milan S. Blake†

Center for Biologics Evaluation and Research, Food and Drug Administration, Washington, DC, USA

New multivalent CRM_{197}-based conjugate vaccines are available for childhood immunization. Clinical studies were reviewed to assess meningococcal group C (MenC) antibody responses following MenC-CRM_{197} coadministration with CRM_{197}-based pneumococcal or *Haemophilus influenzae* type b conjugate vaccines. Infants receiving a total CRM_{197} carrier protein dose of ~50 μg and concomitant diphtheria-tetanus-acellular pertussis (DTaP)-containing vaccine tended to have lower MenC geometric mean antibody titers and continued to have low titers after the toddler dose. Nevertheless, at least 95% of children in the reported studies achieved a MenC serum bactericidal antibody (SBA) titer of ≥1:8 after the last infant or toddler dose. SBA was measured using an assay with a baby rabbit or human complement source. Additional studies are needed to assess long-term antibody persistence and MenC CRM_{197} conjugate vaccine immunogenicity using alternative dosing schedules.

Encapsulated bacteria, including *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* type b (Hib), have historically been causes of invasive disease in young children. Clinical characteristics common to these pathogens are the early onset of nonspecific symptoms, which can rapidly progress to meningitis or fulminant septicemia. A timely clinical diagnosis is difficult, and even with available treatments, neurological impairment occurs among disease survivors. In children with certain underlying medical conditions, such as late complement component deficiencies or sickle cell disease, susceptibility to systemic encapsulated bacterial disease results in significant morbidity and mortality.

After maternal antibody declines, infants have little acquired natural immunity to meningococcal, pneumococcal, and Hib organisms. Invasive disease rates for these pathogens have been highest among young children and emphasize the need for early-childhood vaccination. In the year prior to mass meningococcal C vaccination in the United Kingdom, approximately 45% of the 2,400 meningococcal disease cases occurred among children younger than 1 year old (31). In the United States, peak incidence rates of invasive pneumococcal and Hib disease in the pre-conjugate vaccine era occurred in 6- to 11-month-old infants (3, 11). For each of the three pathogens, development of glycoconjugate vaccines was able to elicit protective immune responses in infants and young children. Additional benefits of conjugate vaccination included indirect effects in unvaccinated populations and induction of immunologic memory.

The number of multivalent CRM_{197}-based conjugate vaccines included in childhood immunization schedules continues to increase. Three meningococcal CRM_{197}-based conjugate vaccines are available for pediatric use. Two monovalent group C-CRM_{197} conjugate vaccines (Meningitec [Wyeth Pharmaceuticals Inc., Pearl River, NY] and Menjugate [Novartis Vaccines and Diagnostics, Siena, Italy]) are available in many countries and are used routinely in infants and toddlers (28, 36). A quadrivalent (A, C, Y, and W135) meningococcal CRM_{197} conjugate vaccine (Menveo; Novartis Vaccines and Diagnostics, Siena, Italy) is recommended in the United States both for children 2 to 10 years old who are at continued risk of developing meningococcal disease and for routine adolescent immunization (10). In many countries, children who are <2 years old also receive concomitant 7- or 13-valent pneumococcal CRM_{197} conjugate vaccine (Prevnar [PCV7] or Prevnar 13 [PCV13], respectively; Wyeth Pharmaceuticals Inc., Pearl River, NY), depending on the vaccine available. Other CRM_{197}-based candidate pneumococcal conjugate vaccines were previously (9-valent) or are currently (15-valent) being evaluated in clinical trials (21, 25). CRM_{197} is a genetically modified nontoxic form of diphtheria toxin. Diphtheria toxoid, derived from the native toxic form of diphtheria toxin, is a component in diphtheria-tetanus-pertussis vaccines.

Coadministration of conjugate vaccines with the same carrier protein can result in decreased, increased, or no effect on vaccine antibody response (1, 8, 46). We reviewed pediatric studies that included coadministration of meningococcal C-CRM_{197} (MenC-CRM) conjugate vaccine with CRM_{197}-based pneumococcal or Hib vaccines to assess the effect of increasing the CRM_{197} carrier protein dose and coadministered diphtheria-containing vaccines on the meningococcal antibody response.

MATERIALS AND METHODS

Cochrane Database, PubMed, Embase, and regional databases in the World Health Organization International Clinical Trials Registry were systematically reviewed for trials among healthy children less than 2 years old that included meningococcal C immunogenicity data when meningococcal CRM_{197} conjugate vaccine was coadministered with or without other CRM_{197}-based conjugate vaccines. Studies in MenC-CRM-immunized toddlers were included if corresponding infant MenC immunogenicity data were available. Routine childhood vaccinations were given according to local-country recommendations. Published data from February 1999 to August 2011 were identified using conjugate, CRM_{197}, meningococcal, serogroup C, hemophilus, haemophilus, and pneumococcal

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Address correspondence to Lucia H. Lee, Lucia.Lee@fda.hhs.gov.
† Deceased.

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as search terms for English language articles. Trial design was not a selection criterion.

The review focused on immunogenicity comparisons in children who received coadministered meningococcal CRM_{197}-based conjugate and nonmeningococcal CRM_{197} conjugate vaccines given as separate injections. The serum bactericidal antibody (SBA) responses reported pertained to children born at a gestational age of ≥37 weeks. Immunogenicity outcomes included SBA geometric mean titers (GMTs) and seroresponse rates of ≥1:8 and, when available, ≥1:128.

RESULTS

Study design characteristics. Of 25 clinical trials identified, 15 studies involving 2,758 MenC-CRM recipients were included in the analysis (4, 8, 14, 15, 19, 20, 24, 29, 32, 39–41, 43, 44, 47). Thirteen studies were randomized trials with a control group or parallel group, and two were prospective cohort studies. Ten of the 25 studies did not include meningococcal C bactericidal antibody results, infant immunogenicity data, or detailed laboratory methods.

Ten of the 15 trials were conducted in the United Kingdom, and 5 trials occurred in Spain, Germany, or North America. MenC bactericidal antibody responses after meningococcal C and coadministered Hib (Hib-CRM) or pneumococcal (7-, 9-, and 13-valent) CRM_{197}-conjugate vaccinations (PCVs) were compared to antibody responses after MenC-CRM conjugate vaccination alone. Bactericidal meningococcal C antibody titers were measured using an SBA assay with a baby rabbit (rSBA) or exogenous human complement (hSBA) source. An rSBA assay was performed by one laboratory for 11 studies (4, 8, 14, 15, 19, 24, 32, 39, 40, 43, 44) and by another laboratory for 2 studies (41, 47). A third laboratory performed hSBA testing for the remaining 2 studies (20, 29). The study outcomes are summarized in Tables 1 and 2. The immunogenicity outcomes are categorized by assay type (rSBA or hSBA), coadministered diphtheria-tetanus-pertussis vaccine, and total administered CRM_{197} dose/per vaccination visit. The CRM_{197} content of each conjugate vaccine is presented in Table 3.

Immunogenicity. In three studies, MenC-CRM and concomitant diphtheria–tetanus–whole-cell pertussis (DTwP) vaccine was administered to all participants (8, 29, 39). The vaccines were given at 2, 3, and 4 months of age, and MenC antibody responses were available for a common time point (after the 3rd vaccination). Increased total CRM_{197} dose, when CRM_{197}-based vaccines were coadministered with DTwP, was not associated with a MenC dose-dependent antibody response. In a dose-ranging study, participants received MenC-CRM dosages given concomitantly with DTwP/Hib-CRM, which amounted to total doses of 50 μg and 30 μg CRM_{197}/vaccination visit, respectively. Participants received three injections of the same vaccine. After the third immunization, the rSBA GMTs were 1,011 (95% confidence interval [CI], 702, 1,455) and 1,103 (95% CI, 804, 1,513), respectively (39). In another study, infants who received MenC-CRM (15 μg CRM_{197}/per dose) without Hib-CRM achieved an rSBA GMT of 808 (95% CI, 630, 1,037) (8). In the third study, participants received MenC-CRM that contained 20 μg or 13 μg of CRM_{197}. The same vaccine was administered for each injection. Postvaccination hSBA GMTs were 629 and 420, respectively, with overlapping 95% confidence intervals (29).

When CRM_{197}-basedvaccines were coadministered with diphtheria-tetanus-acellular pertussis (DTaP) vaccine, a relative decrease in the MenC antibody response was observed (19, 20, 24, 29, 32, 43). Four trials contained study groups in which MenC-CRM (15 μg CRM_{197}) was coadministered with DTwP (8) or a DTaP combination vaccine at 2, 3, and 4 months of age, and the sera were processed by the same laboratory (19, 32, 43). CRM_{197}-based pneumococcal vaccine (PCV7 or PCV9) was given concomitantly to some of the groups who received DTaP vaccine. MenC antibody responses were measured 1 month after the 3rd vaccination. Infants given MenC-CRM and DTwP vaccine achieved an rSBA GMT of 808 (95% CI, 630, 1,037), whereas infants who received 15 to 37 μg of CRM_{197}/dose and DTaP vaccine achieved rSBA GMTs of 291 to 380. However, in a fifth study, the rSBA GMTs observed were similar among infants who received MenC-CRM vaccine with DTwP or DTaP (24). The administered MenC-CRM and testing laboratory in the fifth study differed from those in the first four described studies. Two studies contained study groups for which hSBA GMTs were available for infants who received MenC-CRM and DTwP or DTaP (20, 29). hSBA GMTs after the 3rd vaccination were 420 (95% CI, 311, 566) and 232 (95% CI, 207, 260), respectively. The hSBA assay used in the two studies was performed by the same laboratory. In toddlers who received a 4th MenC-CRM dose with concomitant DTaP or DTwP vaccine, differences in MenC antibody responses were representative of the vaccines coadministered (4, 15, 20, 29, 40, 42).

When total CRM_{197} doses increased from 35 μg to 47 μg, a proportional decrease in the MenC rSBA GMTs was noted in children who received 7-valent or 13-valent pneumococcal CRM_{197}-based vaccine (PCV7 or PCV13) (15). In this study, DTaP combination vaccines were coadministered with MenC-CRM at ages 2 and 4 months, and PCV vaccines were administered at ages 2, 4, and 6 months. After the 2nd MenC-CRM dose, the rSBA GMTs among PCV7- and PCV13-immunized infants were 266 (95% CI, 235, 302) and 191 (95% CI, 168, 218), respectively. As toddlers, rSBA GMTs were 731 (95% CI, 642, 832) and 432 (95% CI, 361, 517), respectively, following immunizations with the same coadministered vaccines received as infants. Other studies in which toddlers had received consecutive doses of simultaneously administered MenC conjugate vaccine and a CRM_{197}-based pneumococcal vaccine showed similar findings (4, 14). In addition, MenC antibody titers were higher in MenC-CRM-primed children who had received a booster dose of meningococcal conjugate vaccine with a different carrier protein than in children who had received a booster dose of meningococcal vaccine with CRM_{197} (4, 14).

Of studies in which MenC rSBA seroresponse rates of ≥1:128 were available, at least 90% of children achieved this antibody titer 1 month after the last infant dose. In all the studies, at least 95% of the children achieved a MenC rSBA or hSBA titer of ≥1:8 1 month after the last infant or toddler dose.

DISCUSSION

At total CRM_{197} carrier protein amounts above 47 μg/dose, a trend toward lower MenC SBA GMTs was observed following simultaneous administration of meningococcal C-CRM_{197} conjugate vaccine and other CRM_{197}-based conjugate vaccines. Carrier dose-related effects were evident when infants received a conjugate vaccine with higher CRM_{197} content and concomitant DTaP combination vaccines. Reduced MenC antibody responses continued to be observed after the toddler dose, which constitutes the dose that contributes to longer disease protection. In the short term, however, at least 95% of the children in all of the reported studies achieved a MenC rSBA or hSBA titer of ≥1:8, measured 30

TABLE 1 Summary of MenC-CRM_{197} studies (rSBA assay)

Reference	Study design/country (trial period)	*n*	MenC vaccination schedule[f]	CRM_{197} content (μg)	CRM_{197}-containing vaccine[e]	rSBA MenC GMT (95% CI)[a]		
						Postinfant dose no. 2	Postinfant dose no. 3	Posttoddler
DTwP + MenC-CRM_{197} vaccine no. 1								
39	Open label, randomized, parallel group/UK (1995–1996)	57	2, 3 ,4	50	MenC-CRM_{197} + PRP-CRM_{197}	766 (500, 1,174)	1,011 (702, 1,455)	
				30	MenC-CRM_{197} + PRP-CRM_{197}	554 (376, 815)	1,103 (804, 1,513)	
8, 40	Open label, randomized, controlled/ UK (2000–2002)	117	2, 3, 4	15	MenC-CRM_{197}		808 (630, 1,037)	
			12					8,519 (5,432, 13,360)
DTaP + MenC-CRM_{197} vaccine no. 1								
15	Double blind, randomized, controlled/Spain (2006–2008)	297	2, 4	47	MenC-CRM_{197} + PCV13	191 (168, 218)		
			15		(PCV13 t 2, 4, 6 mo)			432 (361, 517)
		284	2, 4	35	MenC-CRM_{197} + PCV7	266 (235, 302)		
			15		(PCV7 at 2, 4, 6 mo)		731 (642, 832)	
4, 44	Open label, randomized, parallel group/UK (2006–2010)	119	2, 3 or 2, 4	35	MenC-CRM_{197} + PCV7	229 (176, 298)		
			12–15	20	MenC-PRP-T + PCV7			467 (330, 662)
32	Prospective cohort study/UK (2005)	53	2, 3, 4	35	MenC-CRM_{197} + PCV7		376 (281, 505)	
DTaP + MenC-CRM_{197} vaccine no. 1								
19	Open label, randomized, controlled/ UK (2001–2004)	60–75	2, 3, 4	37	MenC-CRM_{197} + PCV9		291 (208, 407)	
43	Prospective cohort study/UK[b]	54	2, 3, 4	15	MenC-CRM_{197}		380 (275, 526)	
14	Open label, randomized, parallel group/ Spain (2007–2009)	309	2, 4, 6 or 2, 4	–[c]	MenC		OR = 1	
				–[c]	MenC+ PCV7		OR = 0.52 (0.27, 1.0)	
			14–18	–[d]	MenC + PCV7 (no infant PCV7)			OR = 1
				–[d]	MenC + PCV7 (+ infant PCV7)			OR = 0.57 (0.27, 1.2)
47	Open label, randomized, parallel group/Spain (2001–2002)	220–221	2, 4, 6	15	MenC-CRM_{197}		1,373 (1,197, 1,574)	
			3, 5, 7	15	MenC-CRM_{197}		2,257 (1,964, 2,594)	
41	Open-label, randomized, parallel group/Germany (2003–2004)	105	2, 3, 4	15	MenC-CRM_{197}	1,059 (823, 1,363)	1,401 (1,165, 1,684)	
MenC-CRM_{197} vaccine no. 2 + DTwP or DTaP								
24	Open label, nonrandomized, controlled/UK (2001–2002)	50–52	2, 3, 4	15	MenC-CRM_{197}		2,674 (1,807, 3,956)	
				15	MenC-CRM_{197}		2,165 (1,517, 3,089)	

[a] rSBA assays were conducted by one laboratory for 2 studies (41, 47) and by another laboratory for the remaining 11 studies.
[b] Trial dates were not provided; published in 2001.
[c] PCV7 was coadministered with an infant 3-dose MenC-CRM_{197} (n = 152) or 2-dose MenC-TT (n = 157) immunization series. The number of PCV7 doses given depended on the meningococcal vaccine administered. The effect of PCV7 concomitant administration on MenC SBA GMT is reported as an odds ratio (OR).
[d] The MenC SBA GMT was reported as a combined result of all MenC-CRM_{197}-immunized toddlers. Four consecutive doses of the same MenC-CRM_{197} vaccine were administered in each dose cohort.
[e] PRP-CRM_{197}, polyribosylribitol phosphate-MenC-CRM_{197} vaccine. MenC-CRM_{197} vaccines: no. 1, Wyeth Pharmaceuticals Inc., no. 2, Novartis vaccines and Diagnostics.
[f] Months of age.

TABLE 2 Summary of MenC-CRM_{197} studies (hSBA assay)

Reference	Study design/country (trial period)	*n*	MenC vaccination schedule[a]	CRM_{197} content (μg)	CRM_{197}-containing vaccine[b]	hSBA MenC GMT (95% CI)[c]		
						Postinfant dose no. 2	Postinfant dose no. 3	Posttoddler
DTwP + MenC-CRM_{197} vaccine no. 1								
29	Double blind, randomized, controlled/UK (1995–1996)	32	2, 3, 4	20	MenC-CRM_{197}	302 (180, 506)	629 (462, 857)	
		30		13	MenC-CRM_{197}	220 (127, 380)	420 (311, 566)	
		57/25	12	13 /20	MenC-CRM_{197}			2,448 (1,809, 3,311)[d]
DTaP + MenC-CRM_{197} vaccine no. 1								
20	Double blind, randomized, controlled/ Canada (1999–2001)	155	2, 4, 6 15	15	MenC-CRM_{197}		232 (207, 260)	1,344 (1,199, 1,506)

[a] Months of age.
[b] MenC-CRM_{197} vaccine no. 1, Wyeth Pharmaceuticals Inc.
[c] Same laboratory.
[d] The MenC GMT was reported as a combined result of all Men-CRM_{197}-immunized toddlers. Four consecutive doses of the same Men-CRM_{197} vaccine were administered to each dose cohort.

days after the last infant or toddler dose. Carrier protein effects were not observed when DTwP was a coadministered vaccine, which is attributed to an adjuvant effect of the whole-cell pertussis component (51).

Structural and functional characteristics of CRM_{197} and diphtheria toxoid. Although CRM_{197} and diphtheria toxoid are serologically related, their immunogenic properties differ when they are used as carrier or free (unconjugated) proteins (13, 27, 50). Diphtheria toxoid is derived from formaldehyde treatment of diphtheria toxin. CRM_{197} contains a point mutation in fragment A, which alters the active domain and leads to loss of enzymatic activity and its toxic properties. The structural characteristics of CRM_{197} are maintained, since formaldehyde detoxification is not needed during the CRM_{197} manufacturing process and potential cross-linking to peptones can be avoided (7, 35, 38). The physicochemical properties of CRM_{197} are retained as a carrier protein. As a free protein, CRM_{197} appears less immunogenic than diphtheria toxoid (40), possibly due to the inability of CRM_{197} to bind to NAD^+ (2, 33). Formaldehyde inactivation of diphtheria toxin is a process that stabilizes the toxoid, which can improve the immunogenicity of the free protein (9) but potentially results in loss of T helper cell epitopes. Differences in immunogenicity between Hib vaccines using diphtheria toxoid or CRM_{197} carrier proteins, and which were conjugated using different methods, have been observed in comparative clinical trials (13). Similar findings have been noted in comparative trials of meningococcal vaccines conjugated to diphtheria toxoid or CRM_{197} (23). All vaccines elicited adequate immune responses.

Carrier priming. Carrier-induced epitopic suppression, originally a term used to describe immunological interference with a conjugated vaccine antigen in individuals who had high preexisting antibodies to the carrier protein (12, 22), has frequently been used to describe observed antibody interference following the simultaneous administration of two conjugate vaccines with the same carrier protein. Similarly, immunological interference has been observed among conjugate vaccine antigens following coadministration of diphtheria toxoid and CRM_{197} conjugate vaccine (50, 52). However, in contrast to general mechanisms characteristic of carrier-induced epitopic suppression, the presence of antidiphtheria antibodies prior to the first CRM_{197} conjugate vaccination has not been associated with decreased antibody responses to the conjugated vaccine antigen, suggesting that diphtheria toxoid as an immunogen may require more than one dose to prime for T-cell help (34). Lower diphtheria GMTs have been observed following repeated coadministration of CRM_{197}-based conjugate vaccines (15, 19, 47).

TABLE 3 CRM_{197}-based vaccines

Vaccine	CRM content (μg)
Meningococcal C	15
7-Valent pneumococcal	20
9-Valent pneumococcal	22
H. influenzae type b	25
13-Valent pneumococcal	32

Optimizing infant meningococcal C conjugate vaccination schedules. The studies conducted in the United Kingdom were optimal for assessing bactericidal antibody responses concurrent with meningococcal disease surveillance (4, 8, 19, 24, 32, 39, 40, 43, 44). In late 1999, MenC-CRM was administered to infants at 2, 3, and 4 months of age (31). In 2002, both PCV7 and DTaP combination vaccines were included in the childhood immunization schedule. Reduced MenC antibody responses following increases in administered CRM_{197} doses were observed but did not have an overall population effect (9). In 1999 to 2003, an increased number of meningococcal C cases were reported. A change in the immunization to a 2-dose primary infant series and a booster vaccination in the second year of life increased circulating antibody (45), which had declined by 1 year of age (48). Higher and sustained bactericidal titers conferred direct protection on previously vaccinated infants (6), reduced carriage, and provided indirect protection to unimmunized individuals (30, 37, 49). Bactericidal antibody responses were similar when MenC-CRM and PCV7 were given concomitantly at 4-week or 8-week intervals (44). Two-dose infant Men-CRM and PCV7 schedules, with the second dose given at various intervals, have also been evaluated (18). Assessment of MenC immune responses after a 1-dose MenC conjugate infant schedule followed by one subsequent toddler dose is ongoing (5). Use of CRM_{197} and tetanus toxoid (TT) conjugate

meningococcal C vaccine interchangeably has been another option to reduce exposure to CRM_{197} (4, 14).

The studies reported in this review were conducted in Europe or North America. The extent to which reduced MenC responses occurred in these studies might not be generalizable to MenC CRM_{197}-based combination vaccines, combinations of concomitantly administered infant vaccines in other geographic regions, or another dosing schedule. Observed differences in MenC antibody response could be due to the use of two MenC-CRM vaccines with different conjugation methods, the range of carrier protein contained per dose, or varying results reported between laboratories or within the same laboratory.

Although SBA titers measured by the rSBA assay are not directly comparable to titers measured by the hSBA assay, complement-dependent bactericidal activity can be reliably measured by both assays, regardless of the complement source. Interpretation of absolute titers has limitations. Measurement of serum bactericidal antibodies following conjugate vaccination is indicative of direct protection among immunized individuals (17a) but does not account for possible indirect protective effects. If the indirect effects are significant, the impact of reduced MenC antibody titers might not be readily apparent. Lastly, increased levels of circulating bactericidal antibodies have been observed following booster immunization. The extent to which subsequent persistence of bactericidal antibodies is predictive of the duration of protection is not well defined.

Conclusions. The potential for immune interactions between vaccine components highlights the importance of concomitant vaccine evaluations, particularly for conjugate vaccines with a common carrier protein (26). New extended multivalent meningococcal and pneumococcal CRM_{197} conjugate vaccines could be added to an already complex childhood immunization schedule. A 4-valent meningococcal CRM_{197} conjugate vaccine and a 13-valent pneumococcal conjugate vaccine containing 15 to 64 μg and 32 μg of CRM_{197}, respectively, are available for pediatric use (16, 17). Reduced meningococcal C antibody responses related to an increased total CRM_{197} carrier protein dose clearly impact future immunization strategies to prevent meningococcal C disease in young children. Additional studies are needed to assess meningococcal C CRM_{197} conjugate vaccine immunogenicity using alternative dosing schedules, antibody persistence, and carriage effect. Cumulative CRM_{197} effects on meningococcal antibody responses are also important to evaluate in children given three or four PCV7 (cumulative 60 to 80 μg CRM_{197}) infant immunizations and meningococcal CRM_{197} or diphtheria conjugate vaccination in adolescence. Postlicensure surveillance is essential as a continued assessment of meningococcal conjugate vaccine effectiveness.

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